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ning of each regular issue of the PCT Gazette.(54) Title: DEOXOARTEMISININ ANALOGS, PROCESS FOR THEIR PREPARATION, AND ANTICANCER AGENT COM-
PRISING THEM(57) Abstract: The present invention relates to a new deoxoartemisinin dimer and trimer, which have excellent anticancer activity
and lower toxicity and are stable to acids, to a new deoxoartemisinin monomer of intermediate thereof, to preparations thereof, and
to anticancer agents comprising the deoxoartemisinin dimer or trimer.

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DEOXOARTEMISININ ANALOGS, PROCESS FOR THEIR PREPARATION,
AND ANTICANCER AGENT COMPRISING THEM

5

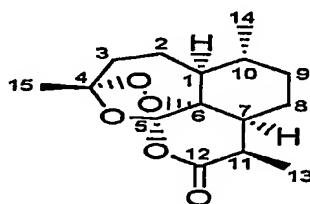
TECHNICAL FIELD

The invention relates to deoxoartemisinin analogs, process for their preparation and anticancer agent comprising them. More particularly, the present invention relates to a
10 new deoxoartemisinin dimer and trimer, which have excellent anticancer activity and lower toxicity and are acid stable, to a new deoxoartemisinin monomer of intermediate thereof, to preparations thereof, and to anticancer agents comprising the deoxoartemisinin dimer or trimer.

15

BACKGROUND ART

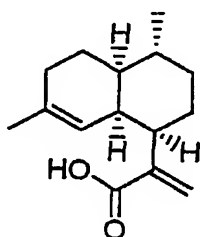
Artemisinin (Qinghaosu)(I), a sesquiterpene lactone endoperoxide, is the first natural trioxane isolated from *Artemisia annua*, L.



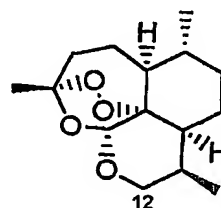
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The artemisinin is of special biological interest because of its outstanding antimalarial activity and outstanding activity against *pneumocystis carinii* and *toxoplasma gondii*. The anti-human immunodeficiency virus (HIV) activity of artemisinin derivatives has been also reported. Artemisinin has been subjected to a number of reviews because of its novel
25 structure and outstanding antimalarial activity. Most first generation C-12 acetal type derivatives are hydrolytically unstable. Also, most semi-syntheses have involved

replacing the C-12 acetal functionality in ether derivatives by less hydrolytically prone functional groups. Recently, however, C-12 non-acetal-type deoxoartemisinin (III) prepared from either artemisinin of formula (I) or artemisinic acid of formula (II) has been reported to show more antimalarial activity than that of artemisinin both *in vitro* and *in vivo* (see Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D., A Short and Stereospecific Synthesis of (+)-Deoxoartemisinin and (-)-Deoxodesoxyartemisinin, *Tetrahedron Lett.*, 1989, 30, 5973-5976 and Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D.; Milhous, W. K., Synthesis and Antimalarial Activity of (+)-Deoxoartemisinin, *J. Med. Chem.*, 1990, 33, 1516-1518).



II



III

Non-acetal-type analogs of deoxoartemisinin recently received attention owing to their better bioavailability, such as acid stability, than acetal-type analogs. Furthermore, evidence that analogs not possessing exo-oxygen at C-12 are less neurotoxic in animal studies than acetal type artemisinin is also emerging and may thus lead to the future abandonment of the currently clinically used acetal-type analogs (e.g., arteether, artemether, artesunate and artelinic acid). After the preparation of 12-n-butyldeoxoartemisinin as the first hydrolytically stable non-acetal type analog containing a C-C bond at C-12 was reported, a series of non-acetal-type derivatives including a few of heteroaryl and unsaturated substituents at C-12 have been prepared (See Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D., A Concise and Stereoselective Synthesis of (+)-12-n-Butyldeoxoartemisinin, *Synlett*, 1990, 743-744 and Chorki, F.; Crousse, B.; Bonnet-Delpon, D.; Begue, J. P.; Brigaud, T.; Portella, C., C-10 Fluorinated Derivatives of Dihydroartemisinin: Difluoromethylene Ketones, *Tetrahedron Lett.*, 2001, 42, 1487-1489).

Although most studies have focused on antimalarial activities, a few research groups have

recently reported on cancer cell toxicity of artemisinin and its related derivatives. (see Woerdenbag, H. J.; Moskal, T. A.; Pras, N.; Maringle, T. M.; ElFeraly, F. S.; Kampinga, H. H.; Konings, A. W. T., Cytotoxicity of Artemisinin-related Endoperoxides to Ehrlich ascites Tumor cells, *J. Nat. Prod.*, **1993**, *56*, 849 and Wu, J-M.; Shan F.; Wu, G-S.; Li, Y.; Ding, J.; Xiao, D.; Han, J-X.; Atassi, G.; Leonce, S.; Caignard, D-H.; Renard, P., Synthesis and Cytotoxicity of Artemisinin derivatives containing Cyanoarylmethyl group, *Eur. J. Med. Chem.*, **2001**, *36*(5), 469-479). Because of their higher rate of cell division, most cancer cells express a higher surface concentration of transferrin receptors than normal cells and have high rates of iron intake. A unique structure bearing endoperoxide could be a trigger for the generation of active oxygen radicals via hemolytic cleavage of the weak oxygen-peroxide bond accelerated by higher ferrous ion concentration of cancer cell, which may mediate for the selective and preferable damage to vital cellular structures of the relatively active cancer cells. Although some dimers of acetal type derivatives of artemisinin have been prepared and show anticancer activities, yields are low and most of them possess either aromatic linkers or still acetal types at the C-12 position, which are neurotoxic, acid unstable, and show low anticancer activities (see Galal, A. M.; Ahmad, M. S.; El-Feraly, F. S., Preparation and Characterization of a New Artemisinin-Derived Dimer, *J. Nat. Prod.*, **1996**, *59*, 917-920; Posner, G. H.; Ploypradith, P.; Parker, M. H.; O'Dowd, H.; Woo, S-H.; Northrop, J.; Krasavin, M.; Dolan, P.; Kensler, T. W.; Xie, S.; Shapiro, Antimalarial, Antiproliferative, and Antitumor Activities of Artemisinin-Derived, Chemically Robust, Trioxane Dimers, *J. Med. Chem.*, **1999**, *42*, 4275-4280; and Ekthawatchai, S.; Kamchonwongpaisan, S.; Kongsaree, P.; Tarnchompoo, B.; Thebtaranonth, Y.; Yuthavong, Y., C-16 Artemisinin Derivatives and Their Antimalarial and Cytotoxic Activities: Synthesis of Artemisinin Monomers, Dimers, Trimers, and Tetramers by Nucleophilic Additions to Artemisitene, *J. Med. Chem.*, **2001**, *44*, 4688-4695).

DISCLOSURE

Surprisingly, we found that deoxoartemisinin dimer and trimer had excellent anticancer activity, by preparing non-acetal-type deoxoartemisinin dimer and trimer having no linker containing C-O bonds, aromatic or unsaturated groups at C-12 position and then testing the

anticancer activity thereof.

Accordingly, it is an object of the present invention to provide a deoxoartemisinin dimer and trimer having excellent anticancer activity.

5

It is another object of the present invention to easily prepare the deoxoartemisinin dimer and trimer in high yield.

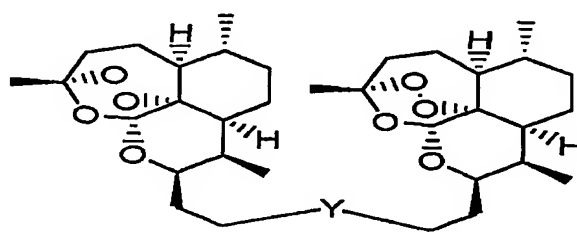
10 It is another object of the present invention to provide anticancer agents comprising the deoxoartemisinin dimer and trimer.


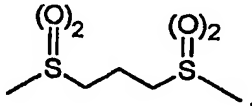
It is another object of the present invention to provide a deoxoartemisinin monomer of intermediate for preparing the deoxoartemisinin dimer and trimer in high yield.

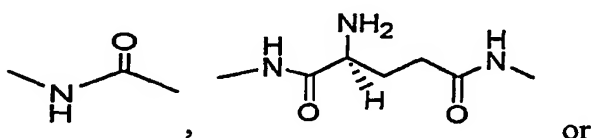
15 It is another object of the present invention to easily prepare the deoxoartemisinin monomer in high yield.

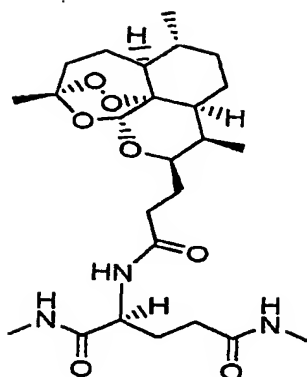
Deoxoartemisinin dimer and trimer of the present invention have the following formula (IV):

20

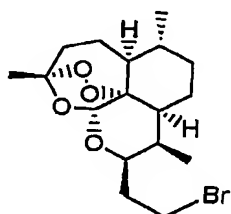


wherein Y is -S-, -SO₂-, , ,

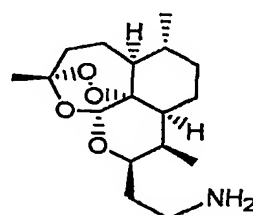




The deoxyartemisinin dimer and trimer of the present invention are prepared from the deoxyartemisinin monomers of formula (V) and (VI), respectively, as shown in Schemes 1, 2 and 3 below.



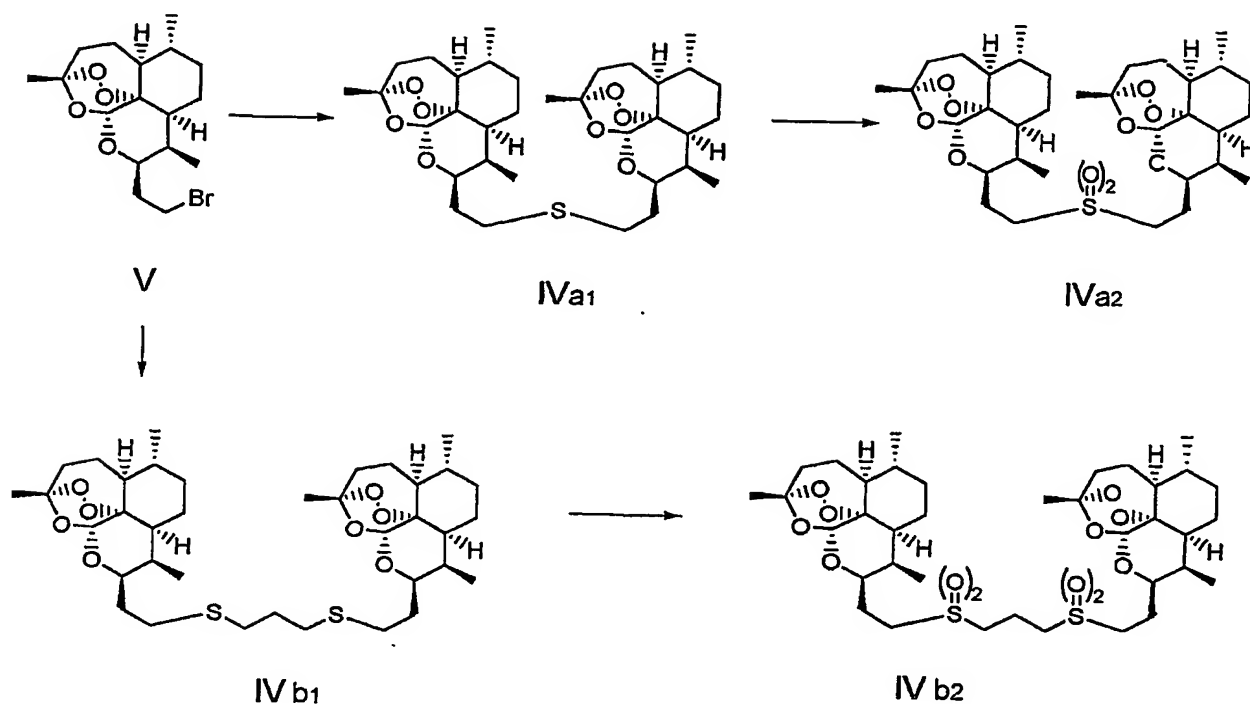
V



VI

The deoxyartemisinin dimer of the present invention having sulfide- or sulfonyl group-bearing linker is prepared by bis-nucleophilic coupling reaction of 12-bromoethyldeoxyartemisinin monomers (V), as shown in Scheme 1 below. Particularly, the deoxyartemisinin dimer (IVa₁) is prepared by reacting 1 mole of sodium sulfide with 2 moles of 12-bromoethyldeoxyartemisinin (V) and dimer (IVa₂) is prepared by oxidizing the dimer (IVa₁) with oxidizing agent such as meta-chloroperoxybenzoic acid (m-CPBA). In a similar fashion, the deoxyartemisinin dimer (IVb₁) is prepared by 1 mole of 1,3-propanedithiol with 2 moles of 12-bromoethyldeoxyartemisinin (V) and the dimer (IVb₂) is prepared by reacting the dimer (IVb₁) with an oxidizing agent such as m-CPBA.

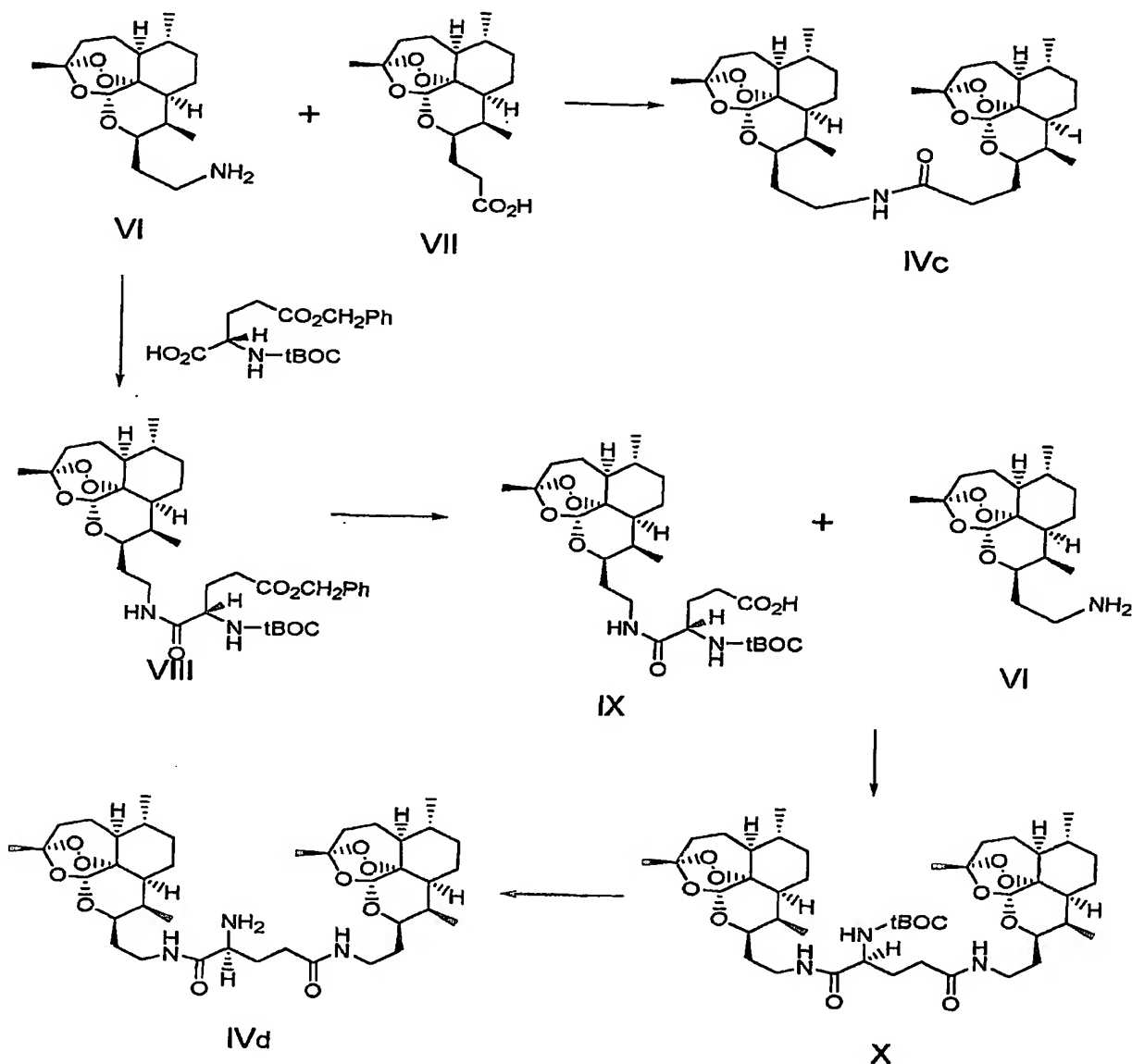
Scheme 1



The deoxoartemisinin dimer of the present invention having amide groups-containing linker is prepared from 12-aminoethyldeoxoartemisinin (VI), as shown in Scheme 2 below. Particularly, the deoxoartemisinin dimer (IVc) is prepared by coupling 12-aminoethyldeoxoartemisinin (VI) with 12-carboxylethyldeoxoartemisinin (VII) in the presence of a catalyst such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDC)/10-hydroxybenzotriazole(HOBt).

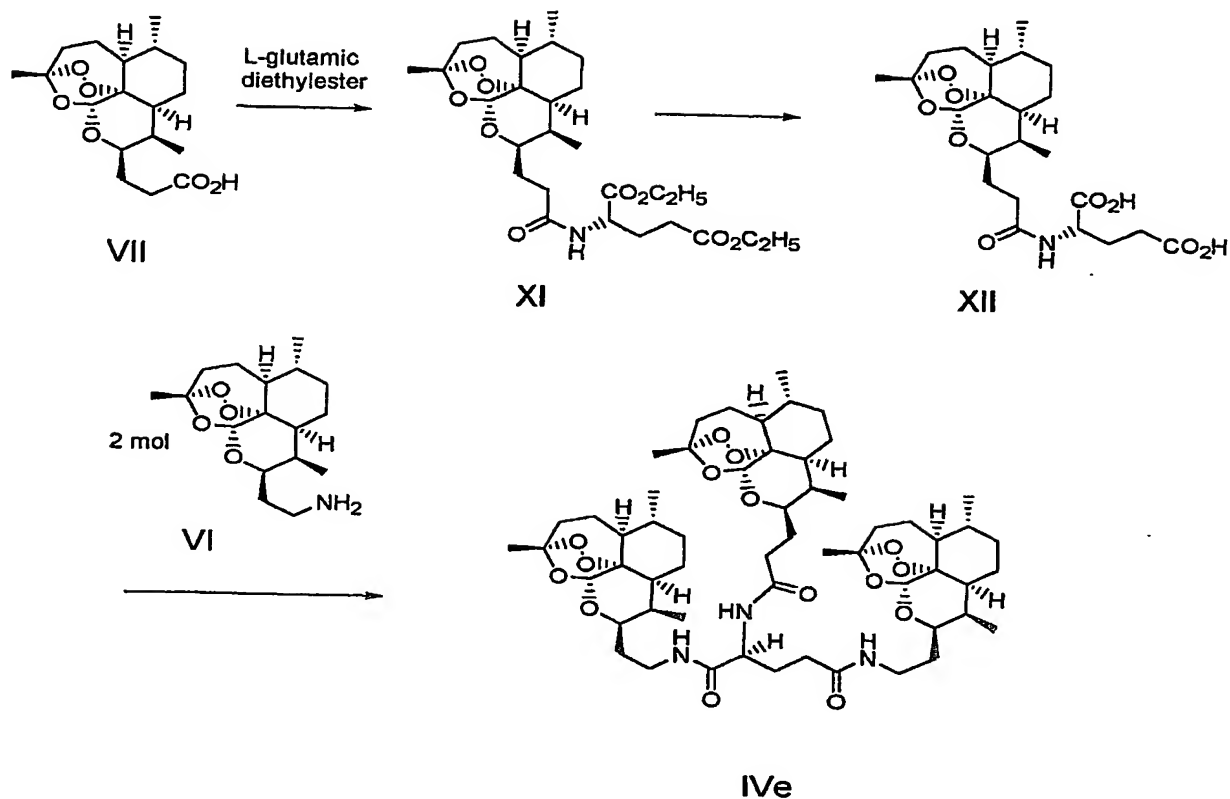
On the other hand, the deoxoartemisinin dimer (IVd) is prepared by directly coupling 12-aminoethyldeoxoartemisinin (VI) with protected glutarate in the presence of a catalyst such as EDC/HOBt, removing the benzyl group of the ester, coupling the resultant product with 12-aminoethyldeoxoartemisinin (VI) in the presence of a catalyst such as EDC/HOBt and then deprotecting the t-BOC protected group of the amino group of the resultant product.

Scheme 2



The deoxoartemisinin trimer (IVe) of the present invention is prepared by coupling
 12-carboxylethyldeoxoartemisinin (VII) with L-glutamic diethylester in the presence of a
 5 catalyst such as EDC/HOBt, hydrolyzing the two ester groups of the product from the
 previous step and then doubly coupling the product from the previous step with 2 moles of
 12-aminoethyldeoxoartemisinin (VI) in the presence of a catalyst such as EDC/HOBt, as
 shown in Scheme 3 below.

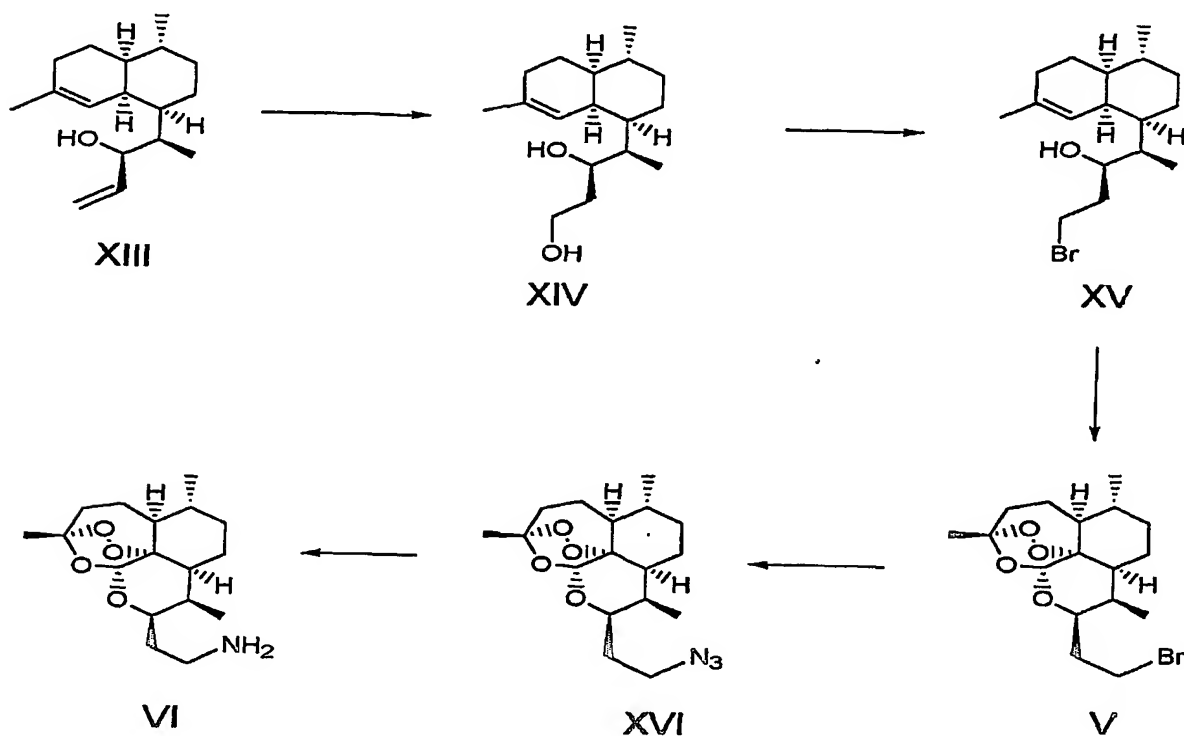
Scheme 3



The novel deoxoartemisinin monomers (V and VI), which are intermediates for preparing the deoxoartemisinin dimer and trimer of the present invention, are prepared from 12-vinyldihydroartemisinin alcohol (XIII), as shown in Scheme 4 below. A 12-vinyldihydroartemisinin alcohol (XIII) can be synthesized from artemisinic acid (II) by a known synthetic route (see Jung et al., A Concise and Stereoselective Synthesis of (+)-12-*n*-Butyldeoxoartemisinin, *Synlett*, 1990, 743-744). Since artemisinin is much more expensive than artemisinic acid and a direct introduction of a C-C bond at C-12 of artemisinin may cause a destruction of the biologically essential endoperoxide, the artemisinic acid is used. Particularly, 12-bromoethyldeoxoartemisinin (V) is prepared by direct hydroborative oxidizing a terminal olefin of 12-vinyldihydroartemisinin alcohol (XIII), brominating the resultant product with $\text{CBr}_4/\text{PPh}_3$, photooxygenative cyclizing the resultant product by a known procedure (see Jung, M et al., *supra*). On the other hand, 12-aminoethyldeoxoartemisinin (VI) is prepared by reacting 12-bromoethyldeoxoartemisinin (V) with sodium azide and then reducing the azide of the

resultant product. In the preparation of 12-bromoethyldeoxoartemisinin (V) and 12-aminoethyldeoxoartemisinin (VI) according to the above-mentioned procedures, 12 β -epimer is obtained exclusively.

5 Scheme 4



The deoxoartemisinin dimer and trimer of the present invention is C-12 non-acetal-type and have no linker containing C-O bonds, aromatic or unsaturated groups at C-12 position, and thus is less neurotoxic, acid stable and have a higher anticancer activity. Therefore, the dimer and trimer of the present invention can be used as an effective anticancer agent for oral administration.

An anticancer agent containing the deoxoartemisinin dimer or trimer of the present invention as an effective constituent can be administered orally (e.g., ingestion or inhalation) or parenterally (e.g., intravenous injection, subcutaneous injection, percutaneous absorption, etc.) and can be prepared in the various form of tablets, capsules, granules, fine subtilae, powders, sublingual tablets, suppositories, ointments, injections,

emulsions, suspensions, drug-treated syrups, etc., depending on its use. The above-mentioned various type of anticancer agents are prepared by a known technique using pharmaceutically acceptable conventional carrier such as excipient, binder, disintegrator, lubricant, antiseptic, antioxidant, isotonic agent, buffer, coating, sweetening,
5 solubilizer, base, dispersion, stabilizer, colorant.

In the preparation of the agent, the content of the compounds of the present invention depends on the type of agents, but preferably ranges from 0.01 to 100% by weight.

10 The dosage of the anticancer agent of the present invention will vary depending on a variety of factors, such as, the kind of mammalian including human to be treated, the severity of the disease and the physician's judgment. Typically, for oral administration, the anticancer agent of the present invention can be administered as an effective constituent in the amount of 0.01 to 50mg per kg body weight per day and, for parenteral
15 administration, in the amount of 0.01 to 10mg per kg body weight per day.

The anticancer agent of the present invention can be administered at one time or at several times per day and its dosage can be varied depending on the severity of the disease and the physician's judgment.

20 The present invention is further described in the following examples. These examples illustrate the invention only and are not to be construed as limiting the scope of the present invention in any way.

EXAMPLES

Example 1: Synthesis of 12-(2'-bromoethyl)deoxoartemisinin (V)

30 (1) Synthesis of 12-(2'-hydroxyethyl)dihydroartemisinin alcohol (XIV).

12-vinyldihydroartemisinyl alcohol (XIII)(568mg, 2.290mmol), prepared by a known procedure (see Jung et al., A Concise and Stereoselective Synthesis of (+)-12-*n*-Butyldeoxoartemisinin, *Synlett*, 1990, 743-744), was slowly added to THF solution containing 0.5M of 9-borobicyclo[3.3.1]nonan(9BBN)(9.1mL, 4.58mmol) under
5 nitrogen atmosphere and the resulting mixture was stirred at room temperature for 30minutes. Then, to this solution was added 30%-H₂O₂/3N-NaOH(1/1, 2mL) and the resulting solution was stirred at room temperature for 1hour. The reaction mixture was extracted with ether (40mL x 2) and washed with saturated NaHCO₃(20mL) and brine (20mL x 2). The extract was dried over MgSO₄, concentrated in vacuo and purified by
10 silica gel column (hexane/ethyl acetate = 1/1 as eluent) to afford 12-(2'-hydroxyethyl)dihydroartemisinyl alcohol (XIV) as a colorless oil (97% yield).

¹H-NMR (CDCl₃, 250MHz) δ5.15(s, 1H, H-5), 4.14(d, 1H, *J*=9.8Hz, H-12), 3.89-3.80(m, 2H, H-2), 2.47(s, 1H), 1.94-1.72(m, 6H), 1.54(s, 3H, CH₃-15), 1.53-1.25(m, 9H), 0.84(d, 3H, *J*=7.3Hz, CH₃-13), 0.87(d, 3H, *J*=6.6Hz, CH₃-14).

¹³C-NMR(CDCl₃, 63MHz) δ135.5, 120.9, 72.0, 62.6, 42.6, 42.5, 39.7, 37.8, 37.6, 36.0, 28.1, 27.0, 26.4, 26.2, 24.1, 20.1, 10.4.

IR(neat) ν_{\max} 3435(OH), 2921, 1647, 1622, 1386, 1124, 1088, 1016 cm⁻¹ MS(EI) *m/z* 266([M⁺]), 248([M⁺]-H₂O), 203([M⁺]-C₂H₅O).

(2) Synthesis of 12-(2'-bromoethyl)dihydroartemisinyl alcohol (XV).

12-(2'-hydroxyethyl)dihydroartemisinyl alcohol (XIV) (399mg, 1.503mmol) was dissolved in dry CH₂Cl₂(20mL) and triphenylphosphine (TPP)(393mg, 1.503mmol) was added.
25 The solution was allowed to stir at 0 °C for 30minutes. The reaction mixture was warmed to room temperature. To this reaction mixture was slowly added CBr₄ (498mg, 1.503mmol). The reaction was allowed to stir at room temperature for 30minutes and was quenched with methanol (10mL). The reaction mixture was extracted with ethyl acetate (20mL x 3) and was washed with brine (20mL x 2). The extract was dried over
30 MgSO₄, concentrated in vacuo and purified by silica gel column (hexane/ethyl acetate = 5/2 as eluent) to afford 12-(2'-bromoethyl)dihydroartemisinyl alcohol (XV)(470mg, 95%)

as a colorless oil.

$^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 5.14(s, 1H, H-5), 4.1(d, 1H, $J=7.5\text{Hz}$, H-12), 3.57-3.52(t, 2H, $J=7.5\text{Hz}$), 2.31(s, 1H), 1.94-1.71(m, 6H), 1.54(s, 3H, CH_3 -15), 1.53-1.25(m, 9H), 0.84(d, 6H, $J=5.0\text{Hz}$, CH_3 -13,14).

$^{13}\text{C-NMR}$ (CDCl_3 , 63MHz) δ 135.5, 120.7, 69.8, 42.8, 42.4, 39.1, 38.9, 37.8, 36.0, 31.9, 28.0, 27.0, 26.4, 26.1, 24.1, 20.1, 10.2.

IR(neat) ν_{max} 3427(OH), 2910, 1726, 1447, 1378, 1259, 992, 908, 734 cm^{-1}

MS(EI) m/z 328($[\text{M}^+]$), 310($[\text{M}^+]-\text{H}_2\text{O}$), 249($[\text{M}^+]-\text{Br}$).

(3) Synthesis of the title compound

12-(2'-bromoethyl)dihydroartemisiny alcohol (XV)(250mg, 0.665mmol) was added to $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$ (1/1, 60mL) containing catalytic Rose Bengal and then was irradiated with white light (500W tungsten lamp) at -23°C for 4hours under oxygen. After completion of the reaction, to the reaction mixture was added saturated NaHCO_3 solution (50mL). This mixture was extracted with diethyl ether (20mL x 3) and then washed with brine (20mL x 2). The extract was dried over MgSO_4 . Solvent was evaporated under reduced pressure and the residue was dissolved in $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$ (9/1, 10mL). This solution was cooled to -40°C . Acidic catalyst trifluoroacetic acid (TFA) was added to this solution and then was allowed to stir at -40°C for 12hours under oxygen. The reaction mixture was quenched with saturated NH_4Cl solution (10mL) and extracted with diethyl ether (20mL x 3). The extract was washed with water (30mL x 2) and brine (30mL x 2), dried over MgSO_4 , concentrated in vacuo and purified by silica gel column (hexane/ethyl acetate = 5/1 as eluent) to afford 12-(2'-bromoethyl)deoxoartemisinin (V)(99mg, 40%) as a white solid.

$[\alpha]_D^{18} = +96.3$ (c 0.1, CHCl_3).

m.p. 94°C .

$^1\text{H-NMR}$ (CDCl_3 , 500MHz) δ 5.26(s, 1H, H-5), 4.33-4.27(m, 1H, H-12), 3.56-3.51(m, 2H, H-2'), 2.60-2.45(m, 2H), 2.30(ddd, 1H, $J=4.1, 3.8, 4.1\text{Hz}$), 2.05-1.89(m, 4H), 1.83-1.48(m,

4H), 1.39(s, 3H, CH₃-15), 1.28-1.22(m, 2H), 0.94(d, 3H, $J=4.8\text{Hz}$, CH₃-13), 0.87(d, 3H, $J=7.4\text{Hz}$, CH₃-14), 0.78(m, 1H).

¹³C-NMR(CDCl₃, 125MHz) δ 103.4, 89.5, 81.2, 73.2, 52.4, 44.4, 37.7, 37.3, 35.8, 34.8, 33.7, 31.6, 30.3, 26.3, 25.0, 20.4, 13.1.

- 5 IR(KBr) ν_{max} 2950, 1451, 1377, 1272, 1117, 1042, 1010, 880(O-O), 756 cm⁻¹
MS(EI) m/z 376(M+2), 342([M+]-O₂).

Example 2: Synthesis of 12-(2'-aminoethyl)deoxoartemisinin (VI).

- 10 (1) Synthesis of 12-(2'-ethyl azide)deoxoartemisinin (XVI).

12-(2'-bromoethyl)deoxoartemisinin (V)(128mg,0.352mmol) was dissolved in DMF(5mL) and sodium azide (45.7mg, 0.704mmol) was added. This mixture was allowed to stir at room temperature for 5hours. To the reaction mixture was added water (30mL). The
15 resulting mixture was extracted with ethyl acetate (50mL x 2) and then washed with brine (40mL x 2). The extract was dried over MgSO₄, concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 5/2 as eluent) to afford 12-(2'-ethyl azide)deoxoartemisinin (XVI)(109mg, 92%) as a colorless oil.

- 20 $[\alpha]_{\text{D}}^{23} = +64.2$ (c 0.47, CHCl₃).

¹H-NMR (CDCl₃, 250MHz) δ 5.28(s, 1H, H-5), 4.31-4.27(m, 1H, H-12), 3.56-3.53(m, 1H), 3.44-3.38(m, 1H), 2.69-2.64(m, 1H), 2.31(ddd, 1H, $J=4.1, 3.8, 4.1\text{Hz}$), 2.03-1.75(m, 5H), 1.67-1.60(m, 3H), 1.40(s, 3H, CH₃-15), 1.33-1.27(m, 3H), 0.96(d, 3H, $J=5.6\text{Hz}$, CH₃-13), 0.87(d, 3H, $J=7.5\text{Hz}$, CH₃-14), 0.82(m, 1H).

- 25 ¹³C-NMR (CDCl₃, 63MHz) δ 103.5, 89.5, 81.3, 72.3, 52.5, 49.8, 44.5, 37.8, 36.9, 34.7, 30.4, 29.5, 26.3, 25.1, 25.1, 20.4, 13.1.

IR(neat) ν_{max} 2927, 2875, 2095(N₃), 1733, 1454, 1377, 1277, 1098, 1011, 880(O-O), 756 cm⁻¹

MS(EI) m/z 337[M+], 305([M+]-O₂).

30

(2) Synthesis of the title compound.

12-(2'-ethylazide)deoxoartemisinin (XVI)(137.9mg, 0.352mmol) was dissolved in dry THF(10mL), cooled to -78 °C and LAH(35.1mg, 0.925mmol) was added. The solution was allowed to stir at -78 °C for 1hour, warmed to -10 °C slowly and allowed to stir at that temperature for 1hour. The reaction mixture was extracted with ethyl acetate (50mL x 2) and washed with brine (40mL x 2). The extract was dried over MgSO₄, concentrated in vacuo, then purified by silica gel column (100% methanol as eluent) to afford 12-(2'-aminoethyl)deoxoartemisinin (VI)(85.4mg, 78%) as a white solid.

$[\alpha]_D^{23} = +38.7$ (c 0.1, CHCl₃).

m.p. 103 °C.

¹H-NMR (CDCl₃, 250MHz) δ 5.32(s, 1H, H-5), 4.29-4.21(m, 1H, H-12), 2.93-2.84(m, 3H), 2.69-2.64(m, 1H), 2.32(ddd, 1H, J=4.0, 3.7, 4.0Hz), 2.05-1.82(m, 3H), 1.80-1.74(m, 2H), 1.62-1.50(m, 2H), 1.40(s, 3H, CH₃-15), 1.32-1.26(m, 4H), 0.96(d, 3H, J=5.7Hz, CH₃-13), 0.87(d, 3H, J=7.5Hz, CH₃-14), 0.83(m, 1H).

¹³C-NMR (CDCl₃, 63MHz) δ 103.5, 89.4, 81.5, 74.2, 52.7, 44.7, 41.0, 37.8, 36.9, 34.8, 33.2, 30.6, 26.5, 25.1, 25.0, 20.5, 13.4.

IR(KBr) ν_{\max} 3365(NH), 2924, 2874, 1663, 1570, 1455, 1377, 1114, 1054, 1011, 944, 877(O-O), 753 cm⁻¹

HRMS(FAB) m/z 312.2175([M+H]⁺, obsd), 311.2097(calcd for C₁₇H₂₉NO₄).

Elemental analysis (C₁₇H₂₉NO₄) C, H, N.

Example 3: Synthesis of 12-(2'-ethylsulfide)deoxoartemisinin dimer (IVa₁).

12-(2'-bromoethyl)deoxoartemisinin (V)(45mg, 0.124mmol) was dissolved in pure ethanol (4mL), allowed to stir at room temperature for 10 minutes and Na₂S(4.8mg, 0.5eq) was added. The reaction mixture was allowed to stir at room temperature for 7hours. This reaction mixture was extracted with ethyl acetate (10mL x 3) and washed with brine (10mL x 2). The extract was dried over MgSO₄, concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 5/1 as eluent) to afford

12-(2'-ethylsulfide)deoxoartemisinin dimer (IVa₁)(58.7mg, 76%) as a colorless oil.

$[\alpha]_D^{25} = +58.7$ (c 0.23, CHCl₃).

¹H-NMR (CDCl₃, 250MHz) δ 5.29(s, 2H, H-5), 4.24-4.19(m, 2H, H-12), 2.91-2.84(m, 2H),
5 2.71-2.70(m, 2H), 2.55-2.49(m, 2H), 2.33(ddd, 2H, J=3.1, 3.5, 4.0Hz), 2.03-1.78(m, 8H),
1.67-1.55(m, 10H), 1.41(s, 6H, CH₃-15), 1.36-1.26(m, 4H), 0.96(d, 6H, J=5.8Hz, CH₃-13),
0.88(d, 6H, J=7.4Hz, CH₃-14), 0.83(m, 2H).

¹³C-NMR (63MHz, CDCl₃) δ 103.6, 89.2, 81.4, 76.8, 75.5, 52.7, 44.8, 37.7, 36.9, 34.8,
30.5, 30.1, 26.5, 25.2, 25.0, 20.6, 13.5.

10 IR(neat) ν_{\max} 2925, 2876, 1617, 1459, 1379, 1119, 1054, 1011, 887(O-O), 735 cm⁻¹
HRMS(FAB) m/z 645.3541([M+Na]⁺, obsd), 622.3539(calcd for C₃₄H₅₄O₈S).

Elemental analysis (C₃₄H₅₄O₈S) C, H, S.

Example 4: Synthesis of 12-(2'-sulfonylethyl)deoxoartemisinin dimer (IVa₂).

15

12-(2'-ethylsulfide)deoxoartemisinin dimer (IVa₁)(28mg, 0.041mmol) was dissolved in dry
CH₂Cl₂(2mL), allowed to stir at room temperature for 10 minutes and m-CPBA(15.7mg,
0.091mmol) was added slowly. The reaction mixture was allowed to stir at room
temperature for 3h and saturated NaHCO₃ solution (3mL) was added. The reaction
20 mixture was extracted with ethyl acetate (10mL x 3) and washed with brine (10mL x 2).
The extract was dried over MgSO₄, concentrated in vacuo, then purified by silica gel
column (hexane/ethyl acetate = 2/1 as eluent) to afford
12-(2'-sulfonylethyl)deoxoartemisinin dimer (IVa₂)(24.6mg, 91%) as a white solid.

25 $[\alpha]_D^{20} = +84.2$ (c 0.44, CHCl₃).

m.p. 98 °C.

¹H-NMR (CDCl₃, 250 MHz) δ 5.30(s, 2H, H-5), 4.25-4.18(m, 2H, H-12), 3.53-3.41(m, 2H,
H-2), 3.05-2.935(m, 2H, H-2), 2.79-2.71(m, 2H), 2.36(ddd, 2H, J=3.2, 3.5, 4.1Hz),
2.17-1.92(m, 6H), 1.88-1.78(m, 4H), 1.69-1.49(m, 8H), 1.39(s, 6H, CH₃-15), 0.97(d, 6H,
30 J=5.7Hz, CH₃-13), 0.92(d, 6H, J=7.6Hz, CH₃-14), 0.89(m, 2H).

¹³C NMR (CDCl₃, 63MHz) δ 134.1, 130.6, 130.1, 128.6, 103.6, 89.4, 81.4, 74.1, 52.5, 44.4,

36.8, 30.5, 26.4, 25.2, 22.4, 20.4, 13.2.

IR(KBr) ν_{\max} 2928, 2876, 1723, 1575, 1449, 1380, 1280, 1123, 1052, 880(O-O), 734 cm^{-1}

HRMS(FAB) m/z 677.3335($[\text{M}+\text{Na}]^+$, obsd), 654.3438(calcd for $\text{C}_{34}\text{H}_{64}\text{O}_{10}\text{S}$).

Elemental analysis ($\text{C}_{34}\text{H}_{64}\text{O}_{10}\text{S}$) C, H, S.

5

Example 5: Synthesis of S,S'-[12-(2'-ethyl)deoxoartemisinin]dithiopropene dimer (IVb₁).

Powdered KOH(12.36mg, 0.22mmol) was added to DMSO(2mL), allowed to stir at room
10 temperature for 1hour and then 1,3-propanedithiol(6.64 μl , 0.054mmol) and
12-(2'-bromoethyl)deoxoartemisinin(V)(41mg, 0.112mmol) was added together. The
reaction mixture was allowed to stir at room temperature for 1hour. The reaction mixture
was extracted with ethyl acetate (30mL x 3) and washed with brine (20mL x 2). The
extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel
15 column(hexane/ethyl acetate = 5/2 as eluent) to afford
S,S'-[12-(2'-ethyl)deoxoartemisinin]dithiopropene dimer(IVb₁)(50.7mg, 65%) as a
colorless oil.

$[\alpha]_D^{24} = +112.3$ (c 0.4, CHCl_3).

20 $^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 5.29(s, 2H, H-5), 4.22-4.17(m, 2H, H-12), 2.83-2.66(m,
10H), 2.41-2.29(m, 2H), 2.05-1.76(m, 10H), 1.66-1.53(m, 10H), 1.41(s, 6H, CH_3 -15),
1.33-1.26(m, 4H), 0.96(d, 6H, $J=5.7\text{Hz}$, CH_3 -13) 0.88(d, 3H, $J=7.5\text{Hz}$, CH_3 -14), 0.83(m,
2H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63MHz) δ 103.6, 89.3, 81.4, 75.3, 52.7, 44.7, 38.8, 37.8, 36.9, 34.8, 32.2,
25 30.5, 30.2, 29.1, 26.5, 25.2, 25.0, 20.5, 13.4.

IR(neat) ν_{\max} 2928, 2873, 1655, 1719, 1452, 1378, 1215, 1120, 1036, 877(O-O), 755 cm^{-1}

HRMS(FAB) m/z 719.3701($[\text{M}+\text{Na}]^+$, obsd) 696.3730(calcd for $\text{C}_{37}\text{H}_{60}\text{O}_8\text{S}_2$).

Elemental analysis ($\text{C}_{37}\text{H}_{60}\text{O}_8\text{S}_2$) C, H, S.

30 **Example 6: Synthesis of S,S'-[12-(2'-ethyl)deoxoartemisinin]disulfonylpropane dimer (IVb₂).**

S,S'-[12-(2'-ethyl)deoxoartemisinin]disulfonylpropane dimer (IVb₂)(22.6mg, 72%) as a white solid was prepared by following the procedure of Example 4, but replacing 12-(2'-ethylsulfide)deoxoartemisinin dimer (IVa₁) with
5 S,S'-[12-(2'-ethyl)deoxoartemisinin]dithiopropene dimer (IVb₁)(29mg, 0.041 mmol) and using 31.1mg of m-CPBA (0.18mmol).

$[\alpha]_D^{25} = +110.4$ (c 0.47, CHCl₃).

m.p. 138 °C.

10 ¹H-NMR (CDCl₃, 250MHz) δ 5.30(s, 2H, H-5), 4.25-4.21(m, 2H, H-12), 3.49-3.43(m, 2H), 3.26(t, 2H, J=7.1Hz), 3.04-2.92(m, 2H), 2.74-2.62(m, 2H), 2.49-2.43(m, 2H), 2.41-2.29(m, 2H), 2.08-1.94(m, 8H), 1.89-1.75(m, 4H), 1.69-1.59(m, 8H), 1.39(s, 6H, CH₃-15), 1.34-1.27(m, 4H), 0.97(d, 6H, J=5.7Hz, CH₃-13), 0.91(d, 6H, J=7.6Hz, CH₃-14), 0.88(m, 2H).

15 ¹³C-NMR(CDCl₃, 63MHz) δ 134.2, 130.6, 130.1, 128.4, 103.6, 89.5, 81.4, 74.1, 52.5, 44.3, 37.7, 36.8, 34.7, 31.3, 30.5, 26.4, 25.2, 20.4, 13.5.

IR(KBr) ν_{max} 2926, 2875, 1720, 1584, 1495, 1387, 1310, 1130, 1052, 877(O-O), 756, 465 cm⁻¹

HRMS(FAB) m/z 783.3538([M+Na]⁺, obsd), 760.3526(calcd for C₃₇H₆₀O₁₂S₂).

20 Elemental analysis (C₃₇H₆₀O₁₂S₂) C, H, S.

Example 7: Synthesis of 12-(2'-amidethyl)deoxoartemisinin dimer (IVc).

25 12-carboxylethyldeoxoartemisinin (VII)(32mg, 0.086mmol), prepared by a known procedure (see Jung, M.; Freitas, A. C. C.; McChesney, J. D.; ElSohly, H. N., A Practical and General Synthesis of (+)-Carboxyalkyldeoxoartemisinins, *Heterocycles*. 1994, 39, 23-29), was dissolved in dry CH₂Cl₂(3mL) and HOBt(38mg, 0.256mmol) and EDC(47mg, 0.256mmol) was added together. The reaction mixture was allowed to stir at room
30 temperature for 30 minutes and 12-(2'-aminoethyl)deoxoartemisinin (VI)(30mg, 0.096mmol) was added. The resulting reaction mixture was allowed to stir at room

temperature for 4 hours. The reaction mixture was extracted with ethyl acetate (20 mL x 3) and washed with brine (10 mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford 12-(2'-aminoethyl)deoxoartemisinin dimer(IVc) in 81 % yield as a colorless oil.

5

$[\alpha]_D^{23} = +111.3$ (c 0.38, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 6.28(s, 1H, NH), 5.30(s, 1H, H-5), 5.29(s, 1H, H-5), 4.33-4.31(m, 1H, H-12), 4.06-4.04(m, 1H, H-12), 3.58-3.56(m, 1H, H-2), 3.28-3.26(m, 1H, H-2), 2.73-2.69(m, 1H), 2.65-2.62(m, 1H), 2.51-2.44(m, 1H), 2.35-2.32(t, 2H, $J=13.5\text{Hz}$), 2.24-2.15(m, 1H), 2.04-1.98(m, 2H), 1.95-1.89(m, 3H), 1.78-1.71(m, 4H), 1.66-1.64(m, 4H), 1.40(s, 6H, CH_3 -15), 1.37-1.24(m, 9H), 0.96(d, 3H, $J=5.3\text{Hz}$, CH_3 -13), 0.95(d, 3H, $J=5.7\text{Hz}$, CH_3 -13), 0.88(d, 3H, $J=7.5\text{Hz}$, CH_3 -14), 0.86(d, 3H, $J=7.5\text{Hz}$, CH_3 -14), 0.84(m, 2H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 173.8, 126.8, 126.1, 118.0, 111.2, 103.7, 103.5, 89.6, 89.0, 81.5, 81.4, 76.4, 74.7, 52.8, 52.5, 44.8, 44.3, 37.8, 37.6, 36.8, 35.1, 34.8, 34.7, 30.7, 30.5, 26.5, 26.4, 25.4, 25.1, 25.0, 20.5, 20.4, 13.7, 13.6.

IR(neat) ν_{max} 3380(NH), 2941, 2877, 1653(C=O), 1545, 1446(C-N), 1379, 1097, 1051, 1013, 915, 878(O-O), 733 cm^{-1}

HRMS(FAB) m/z 634.3995($[\text{M}+\text{H}]^+$, obsd), 633.3877(calcd for $\text{C}_{35}\text{H}_{55}\text{NO}_9$).

20 Elemental analysis ($\text{C}_{35}\text{H}_{55}\text{NO}_9$) C, H, N.

Example 8: Synthesis of 12-[2'-(N-glutamic)- α, β -amide]deoxoartemisinin dimer (IVd).

25 (1) Synthesis of 12-[2'-(N-tBOC-glutamic- γ -benzylester)- α -amide]deoxoartemisinin (VIII).

N-tBOC-L-glutamic acid- γ -benzylester(35mg, 0.11mmol) was dissolved in dry CH_2Cl_2 (5mL) and HOBt(52mg, 0.342mmol) and EDC(63mg, 0.342mmol) was added. 30 The reaction mixture was allowed to stir at room temperature for 30 minutes and 12-(2'-aminoethyl)deoxoartemisinin (VI)(42mg, 0.135mmol) was added. The resulting

reaction mixture was allowed to stir at room temperature for 3 hours. The reaction mixture was extracted with ethyl acetate (20 mL x 3) and washed with brine (10 mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford 12-[2'-(N-tBOC-glutamic- γ -benzylester)- α -amide]deoxoartemisinin (VIII) (71.5 mg, 84%) as a colorless oil.

$[\alpha]^{25}_{\text{D}} = +73.6$ (c 0.47, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 7.36(s, 5H, aromatic H), 7.02(br, 1H, NH), 5.33(s, 1H, H-5), 5.11(s, 2H, benzyl), 4.40-4.39(m, 1H, H-12), 4.29-4.27(m, 1H), 4.15-4.13(m, 1H), 3.58-3.56(m, 1H, H-2), 3.28-3.24(m, 1H, H-2), 2.61-2.43(m, 3H), 2.36-2.14(m, 3H), 2.12-1.98(m, 4H), 1.91-1.65(m, 4H), 1.44(s, 9H, *t*-BOC), 1.42(s, 3H, CH_3 -15), 1.35-1.26(m, 3H), 0.96(d, 3H, $J=5.6\text{Hz}$, CH_3 -13), 0.85(d, 3H, $J=7.6\text{Hz}$, CH_3 -14), 0.82(m, 1H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63 MHz) δ 173.2, 171.5, 155.2, 136.1, 128.8, 128.8, 128.5, 128.5, 128.4, 128.4, 103.8, 89.9, 81.3, 74.4, 66.6, 61.2, 53.1, 52.3, 44.0, 39.4, 37.7, 36.7, 34.6, 30.8, 28.6, 28.5, 28.5, 26.2, 25.1, 25.0, 20.4, 12.6.

IR(neat) ν_{max} 3364(NH), 2932, 2876, 1736(C=O), 1663(C=O), 1538, 1453, 1393, 1249, 1163, 1051, 880(O-O), 702, 610 cm^{-1}

HRMS(FAB) m/z 631.3507 ($[\text{M}+\text{H}]^+$, obsd), 630.3516 (calcd for $\text{C}_{34}\text{H}_{50}\text{N}_2\text{O}_9$).

(2) Synthesis of 12-[2'-(N-tBOC-glutamic acid)- α -amide]deoxoartemisinin (IX).

12-[2'-(N-tBOC-glutamic- γ -benzylester)- α -amide]deoxoartemisinin (VIII) (36 mg, 0.057 mmol) was dissolved in dry THF/ H_2O (1/1, 5 mL) and 1N LiOH (1 mL) was added. The solution was allowed to stir at room temperature for 2 hours. The reaction solution was acidified with 1N HCl, extracted with ethyl acetate (20 mL x 3), and then washed with brine (10 mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford 12-[2'-(N-tBOC-glutamic acid)- α -amide]deoxoartemisinin (IX) (26.8 mg, 87%) as a colorless oil.

$[\alpha]^{25}_{\text{D}} = +66.4$ (c 0.34, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3 , 250 MHz) δ 5.60(d, 1H, $J=7.9\text{Hz}$, NH), 5.33(s, 1H, H-5), 4.34-4.32(m,

1H, H-12), 4.20-4.15(m, 1H), 3.49-3.47(m, 1H, H-2), 3.33-3.31(m, 1H, H-2), 2.66-2.58(m, 1H), 2.48-2.40(m, 2H), 2.30(ddd, 1H, J=2.2, 1.9, 3.7Hz), 2.03-1.73(m, 5H), 1.67-1.61(m, 6H), 1.41(s, 9H, *t*-BOC), 1.38(s, 3H, CH₃-15), 1.33-1.21(m, 3H), 0.95(d, 3H, J=5.1Hz, CH₃-13), 0.84(d, 3H, J=7.4Hz, CH₃-14), 0.81(m, 1H).

5 ¹³C-NMR ((CDCl₃, 63MHz,) δ176.6, 172.2, 103.7, 89.7, 81.4, 76.9, 74.7, 53.4, 52.5, 44.3, 39.1, 37.8, 36.8, 34.7, 30.7, 30.6, 29.8, 28.7, 28.7, 28.7, 28.7, 26.2, 25.2, 25.0, 20.5, 13.0.

IR(neat) ν_{\max} 3352(CO₂H and NH), 2933, 2879, 1714(C=O), 1657(C=O), 1533, 1459, 1379, 1275, 1170, 1053, 914, 882(O-O), 733 cm⁻¹

LCMS(ESI) m/z 540([M+]).

10

(3) Synthesis of 12-[2'-(N-*t*BOC-glutamic)- α , β -amide]deoxoartemisinin dimer (X).

12-[2'-(N-*t*BOC-glutamic acid)- α -amide]deoxoartemisinin (IX)(21mg, 0.039mmol) was dissolved in dry CH₂Cl₂(2mL) and HOBt(22mg, 0.119mmol) and EDC(29mg, 0.119mmol) were added together. The reaction mixture was allowed to stir at room temperature for 30 minutes and 12-(2'-aminoethyl)deoxoartemisinin (VI)(18mg, 0.058mmol) was added. The resulting reaction mixture was allowed to stir at room temperature for 3hours. The reaction mixture was extracted with ethyl acetate (20mL x 3) and washed with brine (10mL x 2). The extract was dried over MgSO₄, concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford 12-[2'-(N-*t*BOC-glutamic)- α , β -amide]deoxoartemisinin dimer (X)(16.6mg, 51 %) as a colorless oil.

20 $[\alpha]^{25}_D = +114.6$ (c 0.46, CHCl₃).

¹H-NMR (CDCl₃, 250MHz) δ6.71(s, 1H, NH), 5.87(s, 1H, NH), 5.34(s, 1H, H-5), 5.33(s, 1H, H-5), 4.34-4.31(m, 2H, H-12), 4.13-4.10(m, 1H), 3.53-3.48(m, 2H), 3.25-3.23(m, 2H), 2.62-2.60(m, 2H), 2.39-2.29(m, 6H), 2.05-1.88(m, 8H), 1.86-1.62(m, 12H), 1.43(s, 9H, *t*-BOC), 1.39(s, 6H, CH₃-15), 1.28-1.25(m, 5H), 0.96(d, 6H, J=5.3Hz, CH₃-13), 0.86(d, 6H, J=7.5Hz, CH₃-14), 0.83(m, 2H).

25 ¹³C-NMR (CDCl₃, 63MHz,) δ173.3, 173.2, 171.5, 171.4, 103.5, 103.4, 89.8, 89.6, 81.4, 81.4, 76.9, 74.3, 52.5, 52.5, 44.4, 44.3, 39.3, 37.8, 36.8, 34.7, 30.8, 28.9, 28.7, 26.4, 25.2, 25.1, 20.5, 14.5, 13.0, 12.8.

IR(neat) ν_{\max} 3379(NH), 2877, 1713(C=O), 1656(C=O), 1545, 1451, 1379, 1268, 1030,

917, 880(O-O), 732 cm^{-1}

LCMS(ESI) m/z 834([M+H]).

(4) Synthesis of the title compound.

5

12-[2'-(N-tBOC-glutamic)- α , β -amide]deoxoartemisinin dimer (X) (28mg, 0.036mmol) was dissolved in dry CH_2Cl_2 (2mL). To this solution was slowly added TFA(4.92mg, 1.2eq) at 0°C for 30 minutes. The resulting reaction mixture was allowed to stir at 0°C for 4hours, extracted with ethyl acetate (20mL x 3) and washed with brine (10mL x 2).
10 The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford 12-[2'-(N-glutamic)- α , β -amide]deoxoartemisinin dimer (IVd)(19mg, 72%) as a colorless oil. The dimer (IVd) is five times more water soluble (5.21 mg/ml) than artemisinin.

$[\alpha]^{25}_{\text{D}} = +120.8$ (c 0.48, CHCl_3).

15 $^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 6.83(s, 1H, NH), 5.74(s, 1H, NH), 5.34(s, 1H, h-5), 5.31(s, 1H, H-5), 4.35-4.33(m, 2H, H-12), 4.15-4.12(m, 1H), 3.53-3.49(m, 2H), 3.27-3.24(m, 2H), 2.64-2.62(m, 2H), 2.32-2.30(m, 2H), 2.15-1.58(m, 12H), 1.53-1.41(m, 10H), 1.39(s, 6H, CH_3 -15), 1.34-1.28(m, 6H), 0.97(d, 6H, $J=5.3\text{Hz}$, CH_3 -14), 0.86(d, 6H, $J=7.5\text{Hz}$, CH_3 -14), 0.82(m, 2H).

20 $^{13}\text{C-NMR}$ (63MHz, CDCl_3) δ 173.4, 173.3, 171.5, 171.3, 103.6, 103.4, 89.8, 89.7, 84.4, 84.3, 76.9, 74.3, 52.6, 52.4, 44.5, 44.3, 39.3, 37.9, 36.7, 34.6, 30.9, 30.8, 28.7, 26.4, 25.2, 25.1, 20.5, 20.4, 14.5, 13.3, 12.7.

IR(neat) ν_{max} 3367(NH), 3098(NH), 2956, 2868, 1689(C=O), 1558, 1446, 1380, 1209, 1137, 998, 887(O-O), 847, 757, 729 cm^{-1}

25 HRMS(FAB) m/z 734.4592([M+H] $^+$, obsd), 733.4513(calc for $\text{C}_{39}\text{H}_{63}\text{N}_3\text{O}_{10}$).

Elemental analysis ($\text{C}_{39}\text{H}_{63}\text{N}_3\text{O}_{10}$) C, H, N.

Example 9: Synthesis of deoxoartemisinin trimer (IVe).

30 (1) Synthesis of N-[12-(β -deoxoartemisinin)propionyl]-L-glutamic diethyl ester (XI).

12-carboxylethyldeoxoartemisinin (VII)(32mg, 0.256mmol) was dissolved in dry CH_2Cl_2 (3mL) and HOBt(38mg, 0.256mmol) and EDC(47mg, 0.256mmol) were added together. The reaction mixture was allowed to stir at room temperature for 30 minutes and L-glutamic diethylester (36mg, 0.171mmol) was added. The resulting reaction mixture was allowed to stir at room temperature for 3hours. The reaction mixture was extracted with ethyl acetate (20mL x 3) and washed with brine (10mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford N-[12-(β -deoxoartemisinin)propionyl]-L-glutamic diethylester (XI) (37.9mg, 84%) as a colorless oil.

$[\alpha]_D^{25} = +44.5$ (c 0.47, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3 , 250MHz,) δ 6.31(d, 1H, $J=7.5\text{Hz}$, NH), 5.29(s, 1H, H-5), 4.63-4.56(m, 1H), 4.23-4.08(m, 5H), 2.75-2.71(m, 1H), 2.50-2.18(m, 7H), 2.04-1.65(m, 9H), 1.40(s, 3H, CH_3 -15), 1.31(s, 3H), 1.28(s, 3H), 1.22-1.15(m, 2H), 0.96(d, 3H, $J=5.8\text{Hz}$, CH_3 -13), 0.88(d, 3H, $J=7.5\text{Hz}$, CH_3 -14), 0.85(m, 1H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63MHz) δ 173.2, 172.3, 103.7, 89.1, 81.5, 76.8, 76.3, 61.9, 61.0, 52.7, 52.0, 44.8, 37.7, 36.8, 34.8, 34.8, 31.2, 30.7, 30.5, 27.8, 26.4, 25.2, 25.0, 20.5, 14.5, 13.5.

IR(neat) ν_{max} 3370(NH), 2939, 2878, 1737(C=O), 1669(C=O), 1533, 1446, 1372, 1054, 1012, 880(O-O), 742 cm^{-1}

MS(FAB) m/z 526.5($[\text{M}+\text{H}]^+$).

Elemental analysis ($\text{C}_{27}\text{H}_{43}\text{NO}_9$) C, H, N.

(2) Synthesis of N-[12-(β -deoxoartemisinin)propionyl]-L-glutamic diacid(XII).

N-[12-(β -deoxoartemisinin)propionyl]-L-glutamic diethylester (XI)(2mg, 0.08mmol) was dissolved in $\text{THF}/\text{H}_2\text{O}$ (1/1, 5mL), 1N LiOH(1mL) was added and then was allowed to stir at room temperature for 2hours. To the reaction mixture was added 1N HCl(1mL). The resulting reaction mixture was extracted with ethyl acetate (20mL x 3) and washed with brine (10mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford N-[12-(β

-deoxoartemisinin)propionyl]-L-glutamic diacid (XII)(30.8mg, 82%) as a colorless oil.

$[\alpha]_D^{26} = +76.8$ (c 0.22, CHCl_3).

m.p. 106 °C.

$^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 8.96(br, 2H, CO_2H), 7.08(d, 1H, $J=6.5\text{Hz}$, NH), 5.34(s, 1H, H-5), 4.62-4.60(m, 1H), 4.12-4.04(m, 1H, H-12), 2.72-2.70(m, 1H), 2.48-2.26(m, 6H), 2.08-1.98(m, 3H), 1.82-1.78(m, 3H), 1.71-1.42(m, 4H), 1.41(s, 3H, CH_3 -15), 1.27-1.21(m, 2H), 0.95(d, 3H, $J=5.3\text{Hz}$, CH_3 -13), 0.87(d, 3H, $J=7.2\text{Hz}$, CH_3 -14), 0.84(m, 1H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63MHz) δ 177.3, 176.6, 175.1, 174.7, 104.3, 89.0, 81.5, 76.9, 52.8, 52.1, 44.9, 37.6, 36.8, 34.8, 34.5, 30.4, 27.1, 26.2, 25.1, 24.9, 21.0, 20.6, 13.7.

IR(KBr) ν_{max} 3346(CO_2H), 2942, 2877, 1728(C=O), 1631(C=O), 1539, 1453, 1381, 1202, 1051, 912, 880(O-O), 732 cm^{-1}

LCMS(ESI) m/z 492 ($[\text{M}+\text{Na}]^+$).

Elemental analysis ($\text{C}_{23}\text{H}_{35}\text{NO}_9$) C, H, N.

(3) Synthesis of the title compound.

N-[12-(β -deoxoartemisinin)propionyl]-L-glutamic diacid (XII)(22mg, 0.047mmol) was dissolved in dry CH_2Cl_2 (2mL) and HOBt(27mg, 0.141mmol) and EDC(31mg, 0.141mmol) were added. The reaction mixture was allowed to stir at room temperature for 30 minutes and 12-(2'-aminoethyl)deoxoartemisinin (VI)(29mg, 0.093mmol) was added. The resulting reaction mixture was allowed to stir at room temperature for 19hours. The reaction mixture was extracted with ethyl acetate (30mL x 3) and washed with brine (20mL x 2). The extract was dried over MgSO_4 , concentrated in vacuo, then purified by silica gel column (hexane/ethyl acetate = 1/2 as eluent) to afford deoxoartemisinin trimer (IVe)(73.3mg, 74%) as a colorless solid.

$[\alpha]_D^{24} = +102.7$ (c 0.41, CHCl_3).

m.p. 138 °C.

$^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 7.13-7.04(m, 2H, NH), 6.83-6.81(m, 1H, NH), 5.32(s, 2H, H-5), 5.29(s, 1H, H-5), 4.39-4.34(m, 3H, H-12), 4.29-4.26(m, 1H), 3.55-3.48(m, 2H), 3.32-3.27(m, 2H), 2.65-2.63(m, 3H), 2.36-2.31(m, 7H), 2.17-1.93(m, 11H), 1.91-1.51(m,

17H), 1.39(s, 9H, CH₃-15), 1.33-1.21(m, 7H), 0.95(d, 9H, J=5.4Hz, CH₃-13), 0.88-0.83(m, 9H, CH₃-14), 0.82(m, 3H).

¹³C-NMR (CDCl₃, 63MHz) δ 174.4, 174.3, 173.2, 173.1, 103.5, 103.4, 89.9, 89.8, 81.5, 81.4, 76.8, 53.2, 53.1, 52.8, 52.6, 52.4, 37.7, 36.8, 34.7, 31.3, 30.8, 30.7, 30.0, 29.1, 26.4, 26.4, 25.1, 20.5, 20.4, 13.2, 13.1.

IR (KBr) u_{max} 3308(NH), 2933, 2875, 1667(C=O), 1535, 1465, 1377, 1102, 1061, 1014, 938, 874(O-O), 751 cm⁻¹

HRMS (FAB) m/z 1056.6392([M+H]⁺, obsd), 1055.6294(calcd for C₅₇H₈₉N₃O₁₅).

Elemental analysis (C₅₇H₈₉N₃O₁₅) C, H, N.

Example 10: Synthesis of 12-(4'-aminobutyl)deoxoartemisinin (VI').

(1) Synthesis of 12-(4'-butyl azide)deoxoartemisinin.

12-(4'-butyl azide)deoxoartemisinin (116.9mg, 92%) was prepared as a colorless oil by following the procedure of Example 2 (1), but replacing 12-(2'-bromoethyl)deoxoartemisinin with 12-(4'-bromobutyl)deoxoartemisinin (137.9mg, 0.352mmol).

[α]_D²⁴ = +71.3 (c 0.1, CHCl₃).

¹H-NMR (CDCl₃, 250MHz) δ 5.29(s, 1H, H-5), 4.19-4.12(m, 1H, H-12), 3.26(t, 2H, J=6.6Hz, H-4), 2.64-2.62(m, 1H), 2.31(ddd, 1H, J=4.1, 3.8, 4.1Hz), 2.04-1.89(m, 2H), 1.68-1.65(m, 2H), 1.63-1.58(m, 7H), 1.40(s, 3H, CH₃-15), 1.34-1.22(m, 4H), 0.96(d, 3H, J=5.7Hz, CH₃-13), 0.86(d, 3H, J=7.5Hz), 0.82(m, 1H).

¹³C-NMR (CDCl₃, 63MHz), δ 103.4, 89.4, 81.4, 75.3, 52.6, 51.7, 44.6, 37.7, 36.9, 34.7, 30.6, 29.3, 29.0, 26.4, 25.2, 25.0, 25.0, 20.5, 13.2.

IR (neat) u_{max} 2927, 2876, 2095(N₃), 1597, 1454, 1379, 1255, 1097, 1012, 946, 881(O-O), 643 cm⁻¹

MS (FAB) 366.4([M+H]⁺).

(2) Synthesis of the title compound.

12-(4'-aminobutyl)deoxoartemisinin (VI')(94.3mg, 79%) was prepared as a white solid by following the procedure of Example 2 (2), but replacing 12-(2'-ethyl azide)deoxoartemisinin with 12-(4'-butyl azide)deoxoartemisinin (128.8mg, 0.352mmol).

$[\alpha]_D^{25} = +49.4$ (c 0.1, CHCl_3).

m.p. 105 °C.

$^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 5.29(s, 1H, H-5), 4.16-4.09(m, 1H, H-12), 2.71-2.66(m, 3H), 2.32(ddd, 1H, $J=4.1, 3.8, 4.1\text{Hz}$), 2.08-2.04(m, 2H), 1.87-1.72(m, 3H), 1.66-1.53(m, 4H), 1.51-1.45(m, 4H), 1.41(s, 3H, CH_3 -15), 1.36-1.24(m, 4H), 0.96(d, 3H, $J=4.3\text{Hz}$), 0.86(d, 3H, $J=7.5\text{Hz}$), 0.83(m, 1H).

$^{13}\text{C-NMR}$ (CDCl_3 , 63MHz), δ 103.5, 89.4, 81.5, 76.0, 52.6, 51.8, 44.8, 38.4, 36.9, 34.8, 31.2, 29.4, 29.0, 26.5, 25.3, 25.2, 25.1, 20.5, 13.3.

IR(KBr) ν_{max} 3378(NH), 2925, 1591, 1454, 1379, 1117, 1038, 1005, 887(O-O), 748 cm^{-1}
HRMS(FAB) m/z 340.2402($[\text{M}+\text{H}]^+$, obsd), 339.2410(calcd for $\text{C}_{19}\text{H}_{33}\text{NO}_4$).

Elemental analysis ($\text{C}_{19}\text{H}_{33}\text{NO}_4$) C, H, N.

Example 11: Synthesis of 12-(4'-aminobutyl)deoxoartemisinin dimer(IVc').

12-(4'-aminobutyl)deoxoartemisinin dimer (IVc')(46mg, 81 %) was prepared as a colorless oil by following the procedure of Example 7, but replacing 12-(2'-aminoethyl)deoxoartemisinin with 12-(4'-aminobutyl)deoxoartemisinin (28mg, 0.096mmol).

$[\alpha]_D^{23} = +104.2$ (c 0.23, CHCl_3).

$^1\text{H-NMR}$ (CDCl_3 , 250MHz) δ 5.68(s, 1H, NH), 5.28(s, 2H, H-5), 4.13-4.03(m, 2H, H-12), 3.25-3.21(m, 2H, H-4), 2.72-2.65(m, 2H), 2.39-2.21(m, 4H), 2.11-1.67(m, 9H), 1.61-1.46(m, 10H), 1.40(s, 6H, CH_3 -15), 1.36-1.22(m, 7H), 0.96(d, 6H, $J=4.6\text{Hz}$, CH_3 -13), 0.89(d, 3H, $J=7.4\text{Hz}$, CH_3 -14), 0.86(d, 3H, $J=7.4\text{Hz}$, CH_3 -14), 0.83(m, 2H).

^{13}C -NMR (CDCl_3 , 63MHz) δ 173.3, 126.5, 126.3, 118.2, 112.5, 103.7, 103.6, 89.4, 89.1, 81.5, 76.9, 76.4, 75.9, 52.8, 52.5, 44.8, 44.3, 37.8, 37.0, 36.4, 36.1, 35.2, 35.1, 34.8, 34.3, 30.6, 30.6, 26.6, 26.4, 25.2, 25.1, 25.1, 25.1, 20.6, 20.5, 13.7, 13.6.

IR(neat) ν_{max} 3388(NH), 2936, 2875, 1650(C=O), 1539, 1452(C-N), 1379, 1216, 1097, 1051, 1005, 873(O-O), 753 cm^{-1}

HRMS(FAB) m/z 662.4173($[\text{M}+\text{H}]^+$, obsd), 661.4190(calcd for $\text{C}_{37}\text{H}_{59}\text{NO}_9$).

Elemental analysis ($\text{C}_{37}\text{H}_{59}\text{NO}_9$) C, H, N.

Example 12: Measurement of anticancer activity.

The anticancer activities of the deoxoartemisinin dimer and trimer of the present invention were measured by a known micro-culture tetrazolium assay (see Carmichel, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B., Evaluation of a Tetrazolium-based Semiautomated Colorimetric Assay: Assessment of Chemosensitivity testing, *Cancer Res.*, 1987, 47, 936-42).

The table 1 shows cytotoxicities of the compounds of the present invention on mouse and human cancer cells *in vitro*.

Table 1

In vitro cytotoxicities (IC_{50} ($\mu\text{g/mL}$))

	P388	EL4	Bewo	HT-29	PANC-1	SKOV3	MCF7	A549
Compound (IVa ₁)	0.40	0.23	14.20	0.24			0.017	
Compound (IVa ₂)	5.60	0.54	1.04	0.38			0.025	
Compound (IVb)	8.40	10.00	8.50	0.38			5.6	
Compound (IVc)	10.40	6.29	7.50	0.69		12.6	0.005	
Compound (IVc')	>20	>20	>20	20.20			>20	
Compound (IVd)	15.60	16.50	>20	6.50			15.3	
Compound (IVe)	0.12	1.07	18.30	0.09	2.69	11.2	0.017	2.45
Adriamycin	0.39	0.67	6.24	0.10			0.12	
Mitomycin	1.50	3.94	0.85	0.02			0.93	1.85

Taxol	2.27	1.34	7.39	0.01	5.76	12.30	0.0001	
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P388: mouse fibroblast leukemia cell

EL4: mouse thymoma cell

Bewo: human choriocarcinoma cell

HT-29: human colorectal adenocarcinoma cell

5 PANC-1: human pancreatic cancer cell

SKOV3: human ovarian carcinoma cell

MCF7: human breast carcinoma cell

A549: human lung cancer cell

- 10 As shown in Table 1, the sulfide dimer (IVa₁) of the present invention exhibited an anticancer activity on the mouse fibroblast leukemia cell (P388), said activity being comparable to that of adriamycin and being at least 4-fold higher than that of mitomycin. Also, the trimer (IVe) of the present invention exhibited an anticancer activity on P388, said activity being at least 3-fold higher than that of adrimycin, at least 12-fold higher than
- 15 that of mitomycin and at least 20-fold higher than that of taxol. The sulfide dimer (IVa₁), sulfone dimer (IVa₂) and trimer (IVe) of the present invention exhibited an anticancer activity on the mouse thymoma cell (EL4), said activity being comparable to that of adriamycin. Most compounds had little anticancer activity on human choriocarcinoma cell (Bewo) but the sulfone dimer (IVa₂) had an anticancer activity on Bewo, which was
- 20 comparable to that of mitomycin and was at least 6-fold higher than that of taxol and adriamycin. The trimer (IVe) of the present invention exhibited an anticancer activity on human colorectal adenocarcinoma cell (HT-29), said activity being comparable to that of adriamycin, and also exhibited an anticancer activity on human pancreatic cancer cell, said activity being at least 2-fold higher than that of taxol. Most compounds had little
- 25 anticancer activity on human ovarian carcinoma cell (SKOV3), while the amide dimer (IVc) and trimer (Ive) of the present invention had an anticancer activity similar to that of taxol. The amide dimer (IVc), sulfide dimer (IVa₁), sulfone dimer (IVa₂) and trimer (IVe) had higher anticancer activity on human breast carcinoma cell (MCF7). Especially, the amide dimer (IVc) of the present invention exhibited an anticancer activity on MCF7, said
- 30 activity being at least 24-fold higher than that of adriamycin and at least 200-fold higher than that of mitomycin. Most of the compounds had less anticancer activity on human

lung cancer cell (A549), while the trimer (IVe) of the present invention had an anticancer activity on A549 similar to that of mitomycin.

5 From the results, it can be seen that the trimer (IVe) of the present invention has a very excellent anticancer activity on most mouse and human cancer cells. Also, it can be seen that the anticancer activity of the dimer of the present invention depends on the length of the linker located between two deoxoartemisinins. In other words, the compounds (IVa₁), (IVa₂) and (IVc), in which a linker has one amide- or one sulfur-centered two ethylene groups, have superior anticancer activity to the compounds (IVa₁), (IVa₂) and (IVc), in
10 which a linker is longer than the length of two ethylene groups.

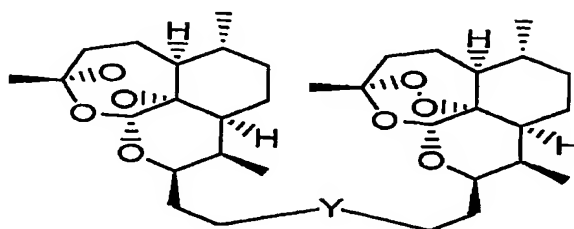
INDUSTRIAL APPLICABILITY

15 The deoxoartemisinin dimer and trimer of the present invention are C-12 non-acetal-type and do not have linker containing C-O bonds, aromatic or unsaturated groups at C-12 positions, and thus are acid stable, less toxic and have higher anticancer activity.

Also, according to the preparation of the present invention, the deoxoartemisinin analog is easily prepared in high yield.

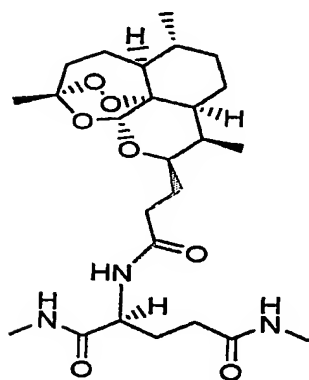
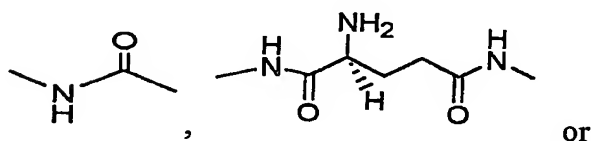
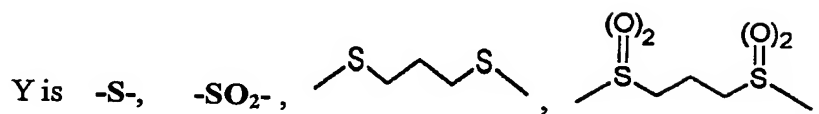
Claims

1. A deoxoartemisinin analog of the following formula:

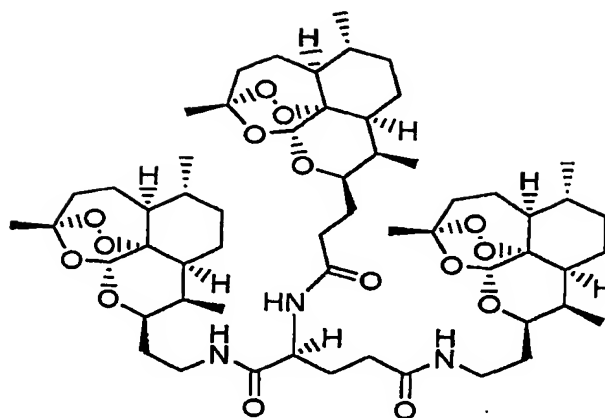


5

wherein



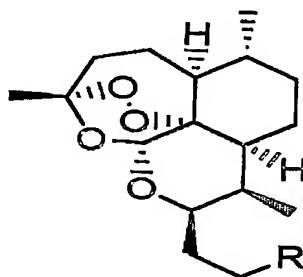
2. A method for preparing deoxoartemisinin trimer of the following formula, said
10 method comprising the steps of: (a) coupling 12-carboxylethyldeoxoartemisinin with L-glutamic diethylester; (b) hydrolyzing two ester groups of the product from said step (a); and (c) doubly coupling the product from said step (b) with 2 moles of 12-aminoethyldeoxoartemisinin:



3. The method as claimed in claim 2, wherein said coupling reaction is carried in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and

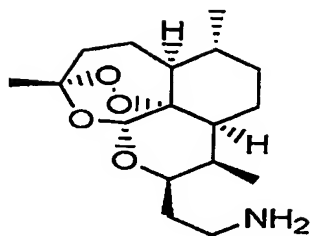
5 10-hydroxybenzotriazole (EDC/HOBt).

4. A deoxyartemisinin analog of the following formula:



wherein R is Br or NH₂.

5. A method for preparing 12-aminoethyldeoxyartemisinin of the following formula, said method comprising the steps of: (a) hydroborative oxidizing a terminal olefin of 12-vinyldihydroartemisinin alcohol; (b) brominating the product from said step (a) with CBr₄/PPh₃; (c) photooxygenative cyclizing the product from said step (b); (d) reacting the product from said step (c) with sodium azide; and (e) reducing an azide group of the product from said step (d):



6. An anticancer agent comprising said deoxoartemisinin analog as claimed in claim

1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2004/000086

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07D 493/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C07D 493/22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and applications for inventions since 1975.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN(CASLINK)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OGNYANOV et. al., 'Synthesis and antimalarial activity of novel C12-ethers of 11-hydroxydihydroartemisinin' In: Dokladi na Bulgarskata Akademiya na Naukite (CODEN: DBANEH. ISSN: 0861-1459), 1993, 46(11), pp. 45-48	4
P, X	JUNG et. al., 'Antitumor activity of novel deoxoartemisinin monomers, dimers, and trimer' In: Journal of Medicinal Chemistry, 13 March 2003, 46(6), pp. 987-994	1 - 6
A	WO 9308195 A1 (The University of Sydney) 29 April 1993, see entire document.	1 - 6

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)	"&" document member of the same patent family
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
08 MAY 2004 (08.05.2004)

Date of mailing of the international search report
12 MAY 2004 (12.05.2004)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2004/000086

Patent document
cited in search report

Publication
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Patent family
member(s)

Publication
date

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21-05-1993